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Influence of extraneous stressors on the physiology of passerine birds: A study at behavioural and neural levels

By

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Chapter 1: Introduction

Birds have evolved over millennia to match their behaviours and physiology to the predictable environmental variations such as seasons and changes in day length in their chosen habitats. They have developed various life history stages (LHS) such as courtship, breeding, parental behaviour, migration, and moult. These highly time-sensitive LHS are present in migrants as well as resident birds, in varying time duration and frequency (Wingfield *et al*., 2017). Passerines belong to the order Passeriformes with approximately 5,000 species that include Zebra finches (*Taeniopygia guttata*) and buntings (*Emberiza spp*.). These two passerine species have generated enormous research interest globally in recent years as: 1. migratory birds hailing from Palearctic region belonging to family Emberizidae called Red-headed bunting (*Emberiza bruniceps*) and 2. resident birds belonging to the family Estrildidae called Zebra finch (*Taeniopygia guttata*).

Whether it is a migratory species, as in bunting, or a resident species as in Zebra finch, birds are faced with several extraneous stressors associated with availability of food, finding a mate for successful reproduction and competition among conspecifics for other resources that lead to alterations in physiology. These stressors eventually underlie survival of the species and the survival strategies adapted.

Stress is the body's response to any change, threat, or pressure imposed on it from outside forces or from within. Depending on the qualitative/quantitative parameters of the stress-inducing stimulus/stressor and its cognitive perception, exposure to stress could lead to expression of normal adaptive behaviours or have a deleterious effect on regulation of homoeostasis (Cockrem, 2007; Wingfield, 2013; Ramenofsky and Wingfield, 2016). Stressors can be any kind of stimulation, internal or external, that trigger the physiological stress response. The state of stress not only activates the hypothalamus-pituitary-adrenal (HPA) axis, but also the reproductive system, i.e., the hypothalamic-pituitary-gonadal (HPG) axis causing an increase in levels of secreted glucocorticoid hormone corticosterone (CORT), the stress hormone (Maddux *et al*., 2015). The three forms of stress that relate to real life stressors faced by birds and therefore taken up for the current study include: 1. Social stress due to social isolation; 2. Environmental stress due to changes in photoperiod and 3. Physiological stress as a result of bacterial infection.

The Zebra finch is a precocial, highly social, monogamous bird model utilised globally to study social and sexual behaviours such as vocalization, courtship, and mate preference (Kniel *et al*., 2015; Katsis *et al*., 2018). Studies suggest that changes in the social environment cause an increase in CORT and have long lasting effects on acoustic expression, social and sexual behaviours (Bölting and von Engelhardt, 2017; Perez *et al*., 2012). However, as most such studies have been conducted in birds before pair bond formation, understanding mate choice

and sexual preferences after separation-induced stress in pair bonded male Zebra finches would provide new insights into the modulation of courtship behaviours as a result of separation/isolation. Despite being monogamous birds, occurrences of re-pairing are witnessed in the wild, either when the mate dies or in instances of separation, while the pair bonded mate is still alive (Schweitzer *et al.*, 2014). Therefore, in our study, we questioned whether pair bonded males would display varied courtship behaviours towards their pair bonded females vis-à-vis new females. Further, we hypothesized whether the period of isolation induces progressive/regressive plasticity changes at the neuronal level in select forebrain areas known to be involved in courtship behaviours/pair bond formation.

Buntings are long-distance Palaearctic-Indian passerines migrating between West Asia (~40°N; breeding grounds) and India (~25°N) where they overwinter between October and late March/April. They follow an annual cyclic pattern and are a perfect model organism to study various reproductive and migratory phenomena at neural and behavioural levels. When held in aviaries under artificial lights and upon experimental modification of day length, they exhibit similar seasonal LHS associated with migration and reproduction (Rani *et al*., 2005; Trivedi *et al*., 2005; Malik *et al*., 2014; Sharma *et al*., 2018). A change in the photoperiod in captivity induces pre-migratory restlessness or *Zugunruhe* in birds, which is manifested in the form of intense night-time activity. During this transition, the avian body undergoes a phase shift from being day-active to night-active, which is accompanied by hyperphagia, fat accumulation and gonadal maturation. Additionally, at the neuronal level, migration-related forebrain and hypothalamic areas witness a phase inversion in neuronal activity, as evidenced by the production of fos protein (Rastogi *et al*., 2011). In the current study, we questioned whether photoperiod-induced changes in physiology and behaviour in the Red-headed bunting are associated with neuro-morphological changes in higher order brain areas, known to be involved in higher order processing, besides migration-related brain areas.

The Red-headed buntings are found to remain in peak migratory condition for only two weeks, with the stress response being activated by the onset of scotophase i.e., the dark period (Landys *et al*., 2004; Mishra *et al*., 2017). As photoperiod is changed from short day to long day, the birds first enter a pre-migratory state during the initial one to two weeks of being in the long day photoperiod, initiate fattening and gonadal recrudescence, but are still day active. Subsequently before entering into spring migratory state, buntings maintained under long day conditions, fatten, show full gonadal maturation and *Zugunruhe*. Under prolonged long day exposure, buntings become photorefractory and stop displaying increased night-time activity. Phase-specific changes in physiology and behaviour have been reported (Rastogi *et al*., 2011). However, we questioned whether there are phase-specific changes in neuronal activity in implicated brain areas that are associated with the phase-specific differences in physiology. Towards this end, behaviour/activity bouts and migratory-related physiological parameters were quantified during the early (after 2 cycles) and late (after 7 cycles) stages of *Zugunruhe*.

The changing neuro-endocrinological parameters resulting from cyclic LHS of buntings have been documented (Rastogi *et al*., 2011; 2013; Surbhi *et al*., 2016) with neuronal activity changes being observed in the brain areas that belong to the visual system of birds and migration-related hypothalamus brain areas (Singh *et al*., 2013). However, no information is available on other brain areas, such as higher order associative areas in the pallium, song control system and limbic brain areas. Therefore, neuronal activity changes during the early and late phases of *Zugunruhe* in these brain areas and in the visual system were quantified using expression of immediate early genes (IEG) *c-fos*.

Avian LHS are highly time-sensitive and exposure to additional stressors such as bacterial infection as a form of physiological stress would be energetically demanding and have detrimental effects on other LHS. The occurrence of infections in any one of these LHS could delay the transition from one stage to the next and put the bird out of sync with its conspecifics in the community or flock and affects reproductive success. Behavioural modifications due to infections commonly manifest as increased somnolence, reduced appetite, locomotor behaviour and body weight (Pedersen *et al*., 2017).

By injecting bacterial lipopolysaccharide (LPS), a common inflammogen and potent mitogen that has been shown to induce immune response, bacterial infection and ensuing immune response can be induced in birds. LPS activates peripheral immune response through a myriad of reaction/activation patterns which include changes in expression of pro- and anti-inflammatory cytokines and activation of brain microglia (Nikodemova and Watters, 2011). Collectively, these reactions are known to increase CORT, thus eliciting the stress response, causing further changes in behaviour and physiology until homeostasis is brought about by the peripheral immune system (Yirmiya and Goshen, 2011; Moyers *et al*., 2015).

Therefore, this study focussed on the effects of extraneous stressors on the physiology of passerines in order to understand how various naturally occurring stressors can affect or influence bird brain and behaviour. To address this aim, the objectives were as follows:

Objectives:

- To establish a sexual preference paradigm to study influence of social stressors.
- To assess the neuromorphological changes that modulates pair bond maintenance.
- To evaluate the neuronal changes in select brain areas implicated in migration, an environmental stressor.
- To delineate activated brain areas by immediate early gene expression as a result of varying life history stages in migratory birds.
- To study behavioural changes during exposure to a peripheral bacterial infection, that is immunological stress, vis-á-vis LPS injections.

Chapter 2: Social isolation-induced effects on male-specific courtship behaviours and dendritic architecture in associative forebrain areas in Zebra finches (*Taeniopygia guttata*)

2.0 Introduction:

Zebra finches (*Taeniopygia guttata*) form long-term pair bonds that last throughout their lifetime and stay continuously paired even when not actively breeding (Prior and Soma, 2015). Maintaining life-long pair bonds is far more beneficial, outweighs the challenges in courting and mating with a new female each season and reduces the selection pressure seen in many seasonal birds. However, pair disruption and re-pairing is said to occur on rare occasions (Zann, 1996; Prior, 2014). Pair disruption and re-pairing are strenuous processes and is reflected physiologically with increased plasma CORT levels (Schweitzer *et al.*, 2014). The males initiate courtship by displaying distinctive courtship behaviours such as allopreening, directed singing, nest box occupancy and clumping. Pair bond formation occurs if both male and female are receptive; courtship develops gradually until a stable pair bond is formed, which usually takes about 7-10 days (Tomaszycki and Adkins-Regan, 2005; Prior, 2014), with days 1-5 considered as early pairing phase and days 10-14 as late pairing phase (Banerjee and Adkins-Regan, 2014).

At the level of the brain, first courtship and sexual imprinting have been shown to differentially express the fos protein in Lateral nido/mesopallium (LNM), the medial Medial nido/mesopallium (MNM) and Arco/nidopallium caudale (ANC) and the secondary visual area Hyperpallium apicale/densocellulare (HAD) (Sadananda and Bischof, 2002). At the neuronal level, stress due to changes in social environment has been reported to affect adult neurogenesis and proliferation in neurogenic brain regions including high vocal centre (HVC) and Hippocampus (Hp) in addition to Caudomedial nidopallium (NCM) and Area X (Brenowitz and Larson, 2015).

The overall aim of this objective was to observe and quantify different courtship behaviours demonstrated by the male Zebra finches on pair bonding, to screen for changes in behaviours post isolation in pair bonded Zebra finch males and to detect differences in behaviors when the isolated male was exposed to new female vis-à-vis the pair bonded female. At the level of the forebrain, the aim was to assess cytoarchitectural changes in neurons by quantifying dendritic intersections and branch points (Rao *et al*., 1994; Ramkumar *et al*., 2012).

2.1 Materials and methods:

2.1.1 Subjects: Adult Zebra finches (*Taeniopygia guttata*) were held in single sexed cages until the experiment began. All birds were maintained on natural photoperiod in the aviary with temperature of approximately 27-30°C and with *ad libitum* access to seed and water. Females served as 'stimulus' birds of which one group was assigned as new female (NF) birds.

2.1.2 Behavioural Analysis: Males and females were randomly assigned to pairs and kept in cages that were visually isolated from other pairs. The birds were left undisturbed for ten days to allow formation of stable pair bond. The recordings were done with Sony camera (Cyber-shot DSC-H300), while maintaining a light intensity of 107 lux and ½ m distance from the cage. The males were then assigned to the following groups as per Tomaszycki and Adkins-Regan (2005): group 1 constituted the pair bonded males (baseline); group 2 were pair bonded males subsequently held visually isolated from females for ten days and then exposed to the same female for one hour (PB) and group 3 were pair bonded males subsequently held visually isolated from females for ten days and then exposed to a new female for one hour (NF). Four courtship behaviours were standardized for analysis, namely clumping, nest box occupancy, allopreening, directed singing. These four parameters were also taken into consideration to confirm pair bonding status. For temporal analysis of behaviours, three days of recordings of over 70 mins each were separated into 10 min bins data.

2.1.3 Golgi Analysis: Male Zebra finches assigned to the following groups were used for Golgi analysis: group 1 were the pair bonded males (baseline), and group 2 were pair bonded males that had been subsequently held visually isolated from females for ten days and then exposed to a new female for one hour (NF). For the Golgi staining, the Rapid Golgi staining technique was used (Patro *et al*., 2013; Rosoklija *et al*., 2003). The slides were visualized using a Leica DM2500 microscope fitted with Digital camera. Camera Lucida drawings of neurons were made at 1000X magnification. Parameters such as no. of dendritic branching points, no. of dendritic intersections, total number of branch points (N_{bp}), terminations (N_t), stem/primary dendrites (N_{pd}; Fukunishi *et al*., 1999) were selected for quantifying neuro-morphological changes.

2.1.4 Statistical analysis: Data are expressed as Mean ± SEM. The data were analyzed statistically by t-test and repeated measures t-test for the behavioural data and one-way ANOVA (repeated measures) for the temporal data. Pearson's correlation was used for correlation between behaviours. t-test was used to analyse the neuronal morphologies in different forebrain areas. The level of significance was defined as $p \leq 0.05$.

2.2 Results:

2.2.1 Behaviour analysis: Baseline courtship behaviours quantified ten days after pairing showed that birds spent higher amount of time either clumping or in nest box with the paired female, while fewer instances allopreening or directed singing. Pearson's correlation between behaviours showed a negative correlation between directed singing vs. clumping ($r = -0.664$, $p=0.036$). Temporal analysis of behaviours across three days (using repeated measures One-way ANOVA) showed no significant differences.

Post isolation males displayed differential courtship behaviours on re-exposure to either their pair bonded female or to a new female. Males showed significant increase in nestbox occupancy ($t_9=7.288$, $p < 0.0001$) when exposed to pair bonded female; whereas clumping

(t_7= 2.093, p = 0.07), allopreening (t_9=0.0051, p > 0.05) and directed singing (t_{10} = 0.66, p > 0.05) were not significantly different. Temporal patterns of post isolation courtship behaviours directed by males at a new female vis-à-vis the pair bonded female were observed with increased nestbox occupancy when exposed to the pair bonded female and increased directed singing towards the new female.

2.2.2 Neuronal morphology and morphometry: Morphometric measures were sampled in different higher order, associative, forebrain areas such as Hyperpallium apicale (HA) and Nidopallium caudale centralis (NCC). Septum served as the control area. Neurons demonstrated no dendritic branching beyond 60µm from the centre of soma. Dendritic intersections were also higher towards the soma and reduced by 80µm with a rare intersection or so at 100µm. In HA and NCC, dendritic branch points and intersections were higher in the pair bonded males than in males that were isolated and exposed to a new female. No significant differences were observed in Septum. A reduction in terminals was observed in HA and NCC neurons of males exposed to a new female post isolation, with no changes total number of branch points or primary dendrites.

2.3 Discussion:

Zebra finch males display various courtship behaviours including directed singing, allopreening, clumping accompanied by courtship dance. The temporal analysis demonstrated no significant differences in baseline courtship behaviours over time, indicating that any variation in the baseline data of the courtship behaviour was not due to the day or time of recording. Of the four, directed singing is one of the initial behaviours that the male displays towards prospective mate. The first sign that a bond has formed is when the pair is in contact, allopreening and found sitting clumped next to each other. Nest building however is associated with copulation, after which partners are inseparable (Zann, 1996; Klatt and Goodson, 2013; Templeton *et al*., 2014). In the current study, we found that males rekindled with their pair bonded mate by spending their time occupying the nestbox, which is one of the pre-parental behaviours, suggesting that social isolation does not erase the memory of the mate. However, post isolation, if the male is exposed to a new female, the males initiate pair bond formation with the new female by initiating directed singing and allopreening. At the neuronal level, dendritic reorganisation was observed in HA and NCC, which have both been identified to be involved in first courtship in males. Studies suggest increased dendritic intersections to be indicative of increased synaptic connections (Rao *et al*., 1994; Ojha and Singh, 2021); therefore, higher number of dendritic branch points and intersections in both the forebrain areas in the pair bonded males as opposed to males that were isolated and exposed to a new female, suggest stabilisation of synaptic connections towards consolidation of memory at a neuronal level for the pair bonded female.

Chapter 3. Neuromorphometric changes associated with photostimulated migratory phenotype in the Palaearctic-Indian migratory Red-headed buntings (*Emberiza bruniceps*)

3.0 Introduction:

Red-headed buntings (*Emberiza bruniceps*) migrating long distances between India (~25°N) in September/October, and West Asia and East Europe (~40°N) in March/April migrate in the night and use photoperiodic cues to regulate their seasonal cycles in migration and reproduction. Throughout the course of a migratory travel, they are exposed to diverse stressors to which they need to adapt to for their survival. Changes in the photoperiod during late spring and early summer trigger the onset of spring migration and regulate physiology and behaviour associated with migration and reproduction to facilitate the long journey to breeding grounds and subsequent breeding upon arrival (Malik *et al*., 2014). Artificially increasing photoperiods in captive birds triggers the same response, which is evidenced by the development of an intense night-time activity or *Zugunruhe* and coincides with the timing and pattern of the actual migration (Berthold 1996; Gwinner 1996; Stevenson and Kumar, 2017; Van Doren *et al*., 2017).

Birds depend on various environmental cues including visual, olfactory, celestial, landmark and geomagnetic orientation for migration. Therefore, in addition to the brain regions implicated in avian migration such as the Suprachiasmatic nucleus (SCN) and Preoptic area (POA); involvement of the visual, olfactory and auditory pathways and the hippocampus is suggested. Activity-dependent plastic changes have been reported to cause increased neuronal recruitment during the development of *Zugunruhe* in hippocampus and hypothalamic nuclei (Hoshooley and Sherry 2007; Rastogi *et al*., 2013). Studies have also shown that changes in the neuronal morphology lead to behavioural memory consolidation (Silva-Gomez *et al*., 2003; Rattenborg and Martinez-Gonzalez, 2011; Taufique *et al*., 2018). The accumulated evidence shows neurons to be highly sensitive to subtle changes in the environment or physiology, which can induce plastic changes as quantified by various dendritic measures and soma size, indicating regressive or progressive plasticity (Amorin and Calisi 2015; Thompson and Brenowitz 2005; Freas *et al*., 2013).

We therefore hypothesized the occurrence of concomitant neuro-morphometric changes in higher-order associative areas with the development of migration and reproduction phenotypes in avian migrants. The migratory Red-headed bunting (*Emberiza bruniceps*) is an ideal experimental system to test this, since different seasonal LHS can be induced by exposing them to appropriate photoperiods (Malik *et al*., 2014).

3.1 Materials and methods:

3.1.1. Birds and housing conditions: Male Redheaded buntings that had been captured from overwintering flocks in late February (~ 25°N) and kept in an outdoor aviary under natural

day lengths for a week were used. These were brought indoors and maintained in a short day (SD) stock room. Birds were then weighed (D0) and transferred individually to activity cages fitted with infrared (IR) sensors to detect activity of the bird throughout the day. After a period of ten SD, a subset of birds was exposed to stimulatory long days (16L:8D) until they showed 7-10 cycles of *Zugunruhe* (LD-Zug), and the other subset continued to be maintained on SD and served as controls. Whereas light intensity of light period was ~200 lux, the dark period was illuminated at ~1 lux. Food and water were provided *ad libitum*, and supplement food prepared from a mixture of boiled egg, cottage cheese, rusk and Vimerol (vitamin supplements) was also provided once a week.

3.1.2 Activity monitoring: Activity monitoring was carried out as Rani *et al*., (2006).

3.1.3 Body weight and fat deposition measurements: Body weights were recorded. Fat score was assayed as per Kumar *et al*., (2001) and Malik *et al*., (2004).

3.1.4 Tissue harvesting: After the LD birds had shown 10.0±2.4 days of *Zugunruhe*, they and Controls held in SD condition were transcardially perfused within 3 h after lights on under deep anesthesia with 0.9% NaCl and 10% formalin. Testes were dissected out and volume calculated as per Kumar *et al*., (2001). Brain was dissected out, post fixed and stained using the Golgi technique as in 2.1.3.

3.1.5 Neuro-morphometry: The soma area and soma perimeter were quantified using LAS-X software with Leica DM2500 microscope. The sum total of all soma were taken and averaged for each animal. Form Factor (FF) was calculated as per Ranjan *et al*., (2014).

3.1.6 Statistical analysis: Data are expressed as Mean ± SEM. Student's t-test tested differences between short and long days. $p \leq 0.05$ was considered as statistically significant.

3.2 Results:

Shift to LD photoperiod altered the 24-h activity pattern. On continued LD exposure, birds began to display intense nocturnal activity or *Zugunruhe*. The activity at night was increased about 200-fold and this continued for the subsequent 8-11 days with significant differences being observed in day and night time activities between LD and SD birds as visualized from actograms. Changes in photoperiod and ensuing *Zugunruhe* induced a significant increase in body weight ($t_6 = 4.584$, $p < 0.01$), fat score ($t_6=6.964$, $p < 0.001$) and testicular volume ($t_6 = 3.508$, $p < 0.05$).

Neuronal soma area and perimeter were analysed. The pyramidal and stellate neurons were observed based on the soma shape. Stellate neurons were ubiquitously distributed, while pyramidal neurons were found in HA, Hippocampus (Hp) and Septum. Highly spinous, multipolar neurons were found in the Entopallium and Nucleus Rotundus. HA had a mixture of pyramidal and stellate neuronal subpopulations, while Hyperpallium Densocellulare (HD) had only stellate neurons.

Soma area and perimeter of the neurons were analyzed in HA, HD, Medial Striatum (MSt) and Hp. Significant increase in soma area ($t_6 = 4.42$, $p < 0.05$) and perimeter ($t_6 = 3.69$, $p < 0.05$) was observed in HA neurons of LD-Zug birds. No significant differences were observed in HD neuronal soma area and perimeter, Hp neuronal soma area and perimeter or MSt neuronal soma area and perimeter. Among hypothalamic brain areas, neuronal soma area and perimeter in Bed Nucleus of Stria Terminalis (BNST) was not significant. Mediobasal hypothalamus (MBH) neurons demonstrated a significant increase in soma area ($t_6 = 13.43$, $p < 0.01$) but not in perimeter in LD-Zug birds. No differences were observed in Paraventricular nucleus (PVN) neuronal soma area and perimeter.

3.3 Discussion:

Brain plasticity in higher-order associative forebrain areas were studied in relation to migratory behaviour in night-migratory passerine Red-headed bunting. In the current study, environmental stress was induced by increasing the photoperiod to replicate spring conditions. Buntings displayed intense night-time activity with corresponding increase in body weight, fat accumulation and gonadal maturation. These behaviours and physiological measures are comparable to the pre-migratory behaviour that buntings display in the wild in preparation for spring migration (Berthold 1996; Malik *et al*., 2014; Sharma *et al*., 2018).

Previous studies have demonstrated the plastic nature of neurons in the song control system (Surbhi *et al*., 2016), hypothalamus (Mishra *et al*., 2018), and hippocampus (Sadananda and Bischof, 2004) in other passerine species, due to seasonal changes, reproduction-associated changes and in spatial memory and navigation respectively. In our study, we observed similar pattern of neuronal plasticity, with the increase in neuronal soma and perimeter in HA neurons and MBH neuronal area in birds that displayed *Zugunruhe*. Therefore, the results indicate that soma perimeter and area measures facilitate quantification of neuronal morphologies in response to stress and corroborate previous studies showing subtle changes in neurons in response to changes in environment and/or physiology (Amorin and Calisi 2015; Thompson and Brenowitz 2005; Freas *et al*., 2013).

Chapter 4. Neuronal activity in brain of Red-headed bunting (*Emberiza bruniceps*) during early and late stages of *Zugunruhe*

4.0 Introduction:

Immediate early genes (IEG) such as *c-fos, zenk* have been used extensively to identify brain regions that are activated during environmentally-induced responses such as courtship, vocalisation and migration-related behaviours. Extensive research on neuro-endocrinology, behaviours and gene expression in Red-headed buntings have established it to be the perfect bird model to study migratory behaviour and subsequent changes (Rastogi *et al*., 2011; 2013; Majumdar *et al*., 2021). *Zugunruhe* expresses the bird's state of readiness to actual migration in response to external or internal cues (Helm, 2006; Stevenson and Kumar, 2017; Van Doren

et al., 2017). Experimentally-or photoperiodically-induced *Zugunruhe* is accompanied by its corresponding physiological changes and inversion in activity from being day-active to night-active birds as seen in the wild. This phase inversion is also seen mirrored at the neuronal level; with the inversion in expression of IEGs in olfactory and visual systems in bird brain (Rastogi *et al*., 2011). In addition to the physiological changes, increased neuronal activity using *c-fos* gene expression has been shown in medial SCN (mSCN) at night during the migratory stage (Rastogi *et al*., 2013). Although, SCN (visual and medial) has been implicated as the neuroanatomical affiliate of the circadian system, Mediobasal hypothalamus (MBH) has been recently recognized to be the regulatory site for photoperiodic seasonal responses in birds, with Infundibular complex (IN) playing a vital part in avian photoperiodic response (Pandey and Bhardwaj, 2016).

Zugunruhe is prominent during scotophase i.e., the dark period (Landys *et al*., 2004). When photoperiod is altered from SD to LD, the birds go from being photosensitive non-migratory to pre-migratory state, before entering into vernal migratory while displaying *Zugunruhe*. During this transition from pre-migratory to migratory state, buntings go through a number of changes including gradual change in the intensity of night-time activity and increase in body weight, fat accumulation and increase in CORT levels (Mishra *et al*., 2018). The birds are said to be in migratory state when they have completed 7 cycles of intense night-time activity. However, we predicted there would be phase-specific differences in the physiology and brain activity of buntings during the early (after 2 cycles) and late (after 7 cycles) stages of *Zugunruhe*.

4.1 Materials and methods:

4.1.1 Subjects: Methodology pertaining to animals, behaviour was same as described in 3.1.1. On day 10, all the birds experienced increase in day length with the change in photoperiod in the chambers to stimulatory LD photoperiod of 13L:11D. Birds were divided into three groups as follows: 1) Long day (LD): exposed to 8 LD 2) *Zugunruhe* day 2 (Zug2): exposed to LD, sacrificed after 2 cycles of *Zugunruhe* and 3) *Zugunruhe* Day 7 (Zug7): exposed to LD, sacrificed after 7 cycles of *Zugunruhe*.

4.1.2 Activity data: As mentioned under 3.1.1.

4.1.3 Body weight and fat deposition using a fat score were measured as mentioned in 3.1.3.

4.1.4 Plasma CORT: The wing vein was punctured and blood collected in heparinized tubes and centrifuged for 10 min at 5000 rpm. at 4°C. Plasma was collected and stored at -20°C until further use. Care was taken to collect blood from birds within 5 mins of being taken out of the cage to avoid time since capture. Sampling was done 2 hours after the birds displayed increased night-time activity. To detect the CORT levels, procedure provided by the manufacturer was followed (Enzo life sciences, ADI 900 097).

4.1.5 All birds were sacrificed after a minimum of 2 hours after lights off to ensure minimal to no neuronal activity observed during staining to be due to day / night transitional effect, since any brain activity occurring due to day / night transition would reach baseline within 2 hours (Mouritsen *et al*., 2005). Night-time perfusions were carried out as described in Rastogi *et al*., (2011). In case of LD group, birds were sacrificed 2 hours after lights off; whereas, in case of birds of Zug2 and Zug7 groups, birds were sacrificed 2 hours after they displayed increased nocturnal locomotor activity consecutively 2 and 7 nights respectively. Testicular volume was measured as mentioned in 3.1.4.

4.1.6 Immunohistochemistry: Brains were dissected and processed as per Sadananda and Bischof (2004). Reference series sections were Nissl-stained by Cresyl Violet stain. Immunohistochemical staining was carried out as described by Sadananda and Bischof (2004).

4.1.7 Statistical analysis: Data are expressed as Mean ± SEM. Statistical analysis of activity patterns and CORT were carried out using paired t-test and One-way ANOVA tests as applicable to test the significance of the results with $p \leq 0.05$.

4.2 Results:

Shift to long photoperiod altered the 24-h activity pattern. On continued long day exposure, birds began to display intense nocturnal activity or *Zugunruhe*. Analysis of the actogram of LD, Zug2 and Zug7 groups, where Zug2 and Zug7 groups displayed increased nocturnal locomotor activity. Physiological data between groups indicate variation in testicular volume ($F_{(2,12)} = 7.006$, $p < 0.01$) and plasma CORT ($F_{(2,7)} = 6.638$, $p < 0.05$). Post-hoc Tukey's multiple comparison test showed significant changes in testicular volume between LD vs. Zug2 ($p < 0.05$) and Zug2 vs. Zug7 ($p < 0.05$) groups as also in plasma CORT LD vs. Zug2 ($p < 0.05$) and Zug2 vs. Zug7 ($p < 0.05$). Body weight and fat scores did not vary.

Differential *c-fos* expression was observed between the three groups in Visual brain areas such as Hyperpallium Apicale (HA), Hyperpallium Densocellulare (HD), and Cluster N; limbic brain areas such as Hippocampus (Hp), and Medial septum (MS); motor control brain areas such as Medial striatum (MSt), and High Vocal Control (HVC), and migratory-related brain areas such as medial Suprachiasmatic Nucleus (mSCN), Mediobasal Hypothalamus (MBH). Olfactory regions such Anterior Olfactory Nucleus (AON), Olfactory Bulb (OB), Lateral Olfactory Cortex (LOC), and Lateral Olfactory Tract (LOT) were constitutively labelled.

4.3 Discussion:

Night-migratory passerine birds such as Red-headed buntings constantly adapt their behaviour and physiology to keep up with the energy demands for the seasonally determined LHS, and for migrating between breeding and wintering grounds to complete their annual cycle. This physiological and behavioural flux is reflected in various neurogenic regions such as SCN (Rastogi *et al*., 2013), song control system (Surbhi *et al*., 2016), and hypothalamus (Mishra *et*

al., 2018) wherein seasonal changes have caused variation in volume of the brain region or in the number or morphology of neurons in these regions. In the wild, the birds are in a non-stop loop of LHS that constitute breeding, moulting, fall migration, wintering, and spring migration, each triggered by changes in temperature, food availability and light conditions (Malik *et al*., 2014). In captivity, these LHS, initiated by experimentally manipulating their living conditions, enable study of mechanisms of each LHS and transitions between them at physiological, neuronal and endocrinological levels.

One such transition is *Zugunruhe*. In the early days of *Zugunruhe* (~Day 2) birds are still in the process of stabilising their daily activity pattern and physiological parameters including body weight, fat accumulation and are highly stressed as seen in their plasma CORT levels. Whereas, by Day 7 the behaviour is stabilised, and birds are in physiological homoeostasis with differential responses at the neuronal level, as observed in *c-fos* expression between the three groups in visual, olfactory, and migration-related brain regions.

Chapter 5. Effect of LPS on behaviour, physiology of passerine birds

5.0 Introduction:

Sickness behaviour can be assessed in Zebra finches by injecting the birds with endotoxin such as LPS. Post LPS exposure, the peripheral immune response is activated in birds, therefore displaying lethargy, reduced food intake and body mass, reduction in levels of luteinizing hormone, increased levels of interleukin (IL)-6 and corticosterone (CORT) (Adelman *et al*., 2010). LPS activates a myriad of immunological events for the body to combat infection. Pro-inflammatory cytokines, including IL-1, tumor necrosis factor alpha and IL-6, produced locally by macrophages and monocytes are associated with the behavioural changes observed in infected animals, a response also known as “sickness behaviour” (Lopes, 2017). Apart from the physiological changes, LPS also induces the activation of microglial cells in the brain which cause a cascade of anti-inflammatory events to reverse the effects of immunological stress (Aidy *et al*., 2014).

In order to fight the infection, most songbirds have increased somnolent behaviour and the transition from wakefulness to sleep follows different behaviours such as active wakefulness, alert wakefulness, drowsiness, front sleep, back sleep, and uni-hemispheric sleep (UHS) (Yadav *et al*., 2017). Songbirds injected with LPS exhibit a decrease in locomotor activity along with reduced appetite, leading to body mass loss. Activities such as hopping, feeding, foraging, perching, drinking, and preening are analysed to observe changes in finches’ daily activity patterns as a result of LPS injection (Ashley *et al*., 2009; Moyers *et al*., 2015). In the current study we aim to tease out the variations in active and somnolent behaviours due to LPS-induced infection and test whether there exist any male-female differences in these behaviours and in immunological parameters.

5.1 Materials and methods:

5.1.1 Subjects: Subjects were male and female wild-type adult Zebra finches (*Taeniopygia guttata*). The birds were divided into four groups (n=4) as follows: male vehicle; male LPS; female vehicle; female LPS.

5.1.2 LPS at 1mg/kg b.w. dissolved in 0.9% NaCl was injected intraperitonially and behavioural recordings were carried out at 3hr, 6hr, 24 hours and 96 hours post injection. The birds were provided with food and water *ad libitum*. Behavioural analysis was carried out by manually scoring time taken and frequency of sickness related behaviours. The recordings were done as described in 2.1.2. Behaviours were primarily categorized as active wakefulness and inactive/immobile. Under active wakefulness behaviours such as hopping, preening, feeding and vocalization were quantified. Under inactive/immobile drowsiness, sleep and time spent awake but immobile were quantified.

5.1.3 Leucocyte quantification: Blood was collected 96hrs post injection. Blood smear preparation and staining to observe differential WBC count was carried out for cytological evaluation, differential counting of WBC and H:L ratio as described by Campbell (2015), and Mitchell and Johns (2008).

5.1.4 Statistics: Data are expressed as Mean ± SEM. One-way ANOVA was carried out to determine statistical significance between groups. The level of significance was defined as $p \leq 0.05$.

5.2 Results:

5.2.1 Behaviours were primarily categorized as active wakefulness and inactive/immobile. Under active wakefulness behaviours hopping was quantified, and under inactive/immobile drowsiness was quantified. Overall, controls spent more time displaying behaviours such as hopping and feeding, whereas LPS-injected birds were found to be inactive, drowsy or resting/immobile while awake and showed varied behaviours at the 3hrs, 6hrs, 24hrs, or 96hrs post injection. LPS-injected birds were found to slowly recover their daily activity with increased hopping at 24hrs post injection, with maximum activity at 96hrs post injection.

5.2.2 The avian blood cells were divided into red blood cells (erythrocytes), white blood cells (leukocytes) and thrombocytes, which are homologous to mammalian platelets. Avian erythrocytes are nucleated. Avian leukocytes are categorized into granulocytes (heterophils, eosinophils, and basophils), mononuclear cells (lymphocytes and monocytes) and thrombocytes. Differential leucocyte count demonstrated differences in the number of granulocytes and mononuclear cells between groups. Further calculation of H/L ratio showed higher H/L ratios in controls as opposed to birds injected with LPS. Two-way ANOVA of LPS vs. Veh in females and males showed significant differences as a result of the treatment ($F_{(1,11)} = 45.20$, $p < 0.001$), but no significant difference due to sex of the birds ($F_{(1,11)} = 2.314$

$p > 0.05$). Post hoc Bonferroni post tests indicated significant differences in H/L ratio between treated and control females ($p < 0.05$) and treated and control males ($p < 0.001$).

5.3 Discussion:

Infections or immunological challenges for birds are stressful with survival pressures, as the body has to divert all the energy away from important LHS such as breeding, migration, courtship to activate an immune response and subsequently maintain homeostasis. Birds have been shown to also display pronounced circadian variation in body temperature and body weight in response to LPS injection, manifested by hypothermia during the day, but fever at night (Sköld-Chiriac *et al*., 2015). The trade-off between their daily activity pattern and immune function is evident with the increase in leucocytes and decrease in daily activities such as hopping, preening and vocalisation. The H:L ratio is a widely used indicator of physiological stress, with changes in the H:L ratio found to be associated with increases in CORT (Campbell, 2015). Similar results were found in the current study indicating that the birds were in physiological stress even at 96 hrs post injection.

Chapter 6. Conclusion

Passerines constitute about 60% of the avian population and are known to be intuitive in predicting and escaping from natural calamities. In the current study, we reiterated the enhanced sensitivity that birds display to external stressors, using variations in social, environmental and immunological statuses as stressors. Physiological, behavioural and neuronal studies in the two passerine species studied revealed similar adaptations during stress response, irrespective of whether the bird was resident or migratory in nature.

Stress due to social isolation and mate separation had an effect on the courtship behaviour of male Zebra finches and these changes were found to be reflected as dendritic modifications in courtship-related brain areas. Environmental stress due to changes in photoperiod revealed concomitant changes in neuromorphology, and expression of *c-fos* and activity patterns in Red-headed buntings. Corresponding changes in behaviour and neurons both in resident and migratory birds, suggests that neuronal plasticity, whether regressive or progressive occurs in higher order associative forebrain areas in addition to neurogenesis occurring in neurogenic regions such as the hippocampus and song control regions in adult birds. Physiological stress as a result of LPS injection showed a drastic reduction in daily activities such as hopping and vocalisation. Birds modulate behaviour as an adaptive tool to cope with the energetic demands during stress response, as seen in increase in leucocytes in association with changes in daily activity pattern when birds were injected with LPS. Overall, we noted that changes in behaviour, physiology and neuronal activity were inter-connected and inter-dependent, and peripheral effects do result in central changes and vice-versa.

7.0 References:

Adelman, J. S., Bentley, G. E., Wingfield, J. C., Martin, L. B., s Hau, M. (2010). Population differences in fever and sickness behaviors in a wild passerine: a role for cytokines. *The Journal of Experimental Biology*, *213*, 4099–4109.

Aidy, S. El, Dinan, T. G., & Cryan, J. F. (2014). Immune modulation of the brain-gut-microbe axis. *Frontiers in Microbiology*, *5*, 3–6.

Amorin, N., & Calisi, R. M. (2015). Measurements of neuronal soma size and estimated peptide concentrations in addition to cell abundance offer a higher resolution of seasonal and reproductive influences of GnRH-I and GnIH in European starlings. *Integrative and Comparative Biology*, *55*, 332-342.

Ashley, N. T., Hays, Q. R., Bentley, G. E., & Wingfield, J. C. (2009). Testosterone treatment diminishes sickness behavior in male songbirds. *Hormones and Behavior*, *56*, 169–176.

Banerjee, S. B., & Adkins-Regan, E. (2014). Same-sex partner preference in adult male zebra finch offspring raised in the absence of maternal care. *Animal Behaviour*, *92*, 167-173.

Berthold, P. (1996). *Control of bird migration*. Springer Science & Business Media.

Bölting, S., & Von Engelhardt, N. (2017). Effects of the social environment during adolescence on the development of social behaviour, hormones and morphology in male zebra finches (*Taeniopygia guttata*). *Frontiers in Zoology*, *14*, 1–18.

Brenowitz, E. A., & Larson, T. A. (2015). Neurogenesis in the adult avian song-control system. *Cold Spring Harbor Perspectives in Biology*, *7*, 1–24.

Campbell, T. W. (2015). Evaluation of the Blood Film. *Veterinary Clinics of North America - Exotic Animal Practice*. Elsevier Inc.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*, 169-178.

Freas, C. A., Roth, T. C., LaDage, L. D., & Pravosudov, V. V. (2013). Hippocampal neuron soma size is associated with population differences in winter climate severity in food-caching chickadees. *Functional Ecology*, *27*, 1341-1349.

Fukunishi, Y., Nagase, Y., Yoshida, A., Moritani, M., Honma, S., Hirose, Y., & Shigenaga, Y. (1999). Quantitative analysis of the dendritic architectures of cat hypoglossal motoneurons stained intracellularly with horseradish peroxidase. *Journal of Comparative Neurology*, *405*, 345–358.

Gwinner, E. (1996). Circadian and circannual programmes in avian migration. *Journal of Experimental Biology*, *199*, 39-48.

Helm, B. (2006). Zugunruhe of migratory and non-migratory birds in a circannual context. *Journal of Avian Biology*, *37*, 533–540.

Hoshooley, J. S., & Sherry, D. F. (2007). Greater hippocampal neuronal recruitment in food-storing than in non-food-storing birds. *Developmental Neurobiology*, *67*, 406-414.

Katsis, A. C., Davies, M. H., Buchanan, K. L., Kleindorfer, S., Hauber, M. E., & Mariette, M. M. (2018). Prenatal exposure to incubation calls affects song learning in the zebra finch. *Scientific Reports*, *8*, 1-10.

Klatt, J. D., & Goodson, J. L. (2013). Sex-specific activity and function of hypothalamic nonapeptide neurons during nest-building in zebra finches. *Hormones and Behavior*, *64*, 818-824.

Kniel, N., Schmitz, J., & Witte, K. (2015). Quality of public information matters in mate-choice copying in female Zebra finches. *Frontiers in Zoology, 12*, 1-8.

Kumar, V., Singh, S., Misra, M., & Malik, S. (2001). Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting (*Emberiza melanocephala*). *Journal of Experimental Biology*, *204*, 2843-2848.

Landys, M. M., Wingfield, J. C., & Ramenofsky, M. (2004). Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli*. *Hormones and Behavior*, *46*, 574–581.

Lopes, P. C. (2017). Why are behavioral and immune traits linked?. *Hormones and Behavior*, *88*, 52-59.

Maddux, S. L., Cristol, D. A., Varian-Ramos, C. W., & Bradley, E. L. (2015). The effect of mercury on baseline corticosterone in a breeding songbird. *Bulletin of Environmental Contamination and Toxicology*, *94*, 135-139.

Majumdar, G., Yadav, G., Malik, S., Rani, S., Balthazart, J., & Kumar, V. (2021). Hypothalamic plasticity in response to changes in photoperiod and food quality: An adaptation to support pre-migratory fattening in songbirds? *European Journal of Neuroscience*, *53*, 430–448.

Malik, S., Rani, S., & Kumar, V. (2004). Wavelength dependency of light-induced effects on photoperiodic clock in the migratory blackheaded bunting (*Emberiza melanocephala*). *Chronobiology International*, *21*, 367-384.
Malik, S., Singh, S., Rani, S., & Kumar, V. (2014). Life at a different pace: Annual itineraries are conserved in seasonal songbirds. *Journal of Biosciences*, *39*, 485–491.
Mishra, I., Agarwal, N., Rani, S., & Kumar, V. (2018). Scotostimulation of reproductive neural pathways and gonadal maturation are not correlated with hypothalamic expression of deiodinases in subtropical spotted munia. *Journal of Neuroendocrinology*. *30*, e12627.
Mishra, I., Singh, D., & Kumar, V. (2017). Daily levels and rhythm in circulating corticosterone and insulin are altered with photostimulated seasonal states in night-migratory blackheaded buntings. *Hormones and Behavior*, *94*, 114–123.
Mitchell, E. B., & Johns, J. (2008). Avian Hematology and Related Disorders. *Veterinary Clinics of North America - Exotic Animal Practice*, *11*, 501–522.
Mouritsen, H., Feenders, G., Liedvogel, M., Wada, K., & Jarvis, E. D. (2005). Night-vision brain area in migratory songbirds. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 8339–8344.
Moyers, S. C., Kosarski, K. B., Adelman, J. S., & Hawley, D. M. (2015). Interactions between social behaviour and the acute phase immune response in house finches. *Behaviour*, *152*, 2039–2058.
Nikodemova, M., & Watters, J. J. (2011). Outbred ICR/CD1 mice display more severe neuroinflammation mediated by microglial TLR4/CD14 activation than inbred C57BL/6 mice. *Neuroscience*, *190*, 67–74.
Ojha, K., & Singh, K. P. (2021). Seasonal plasticity in the morphology and cytoarchitecture of the hippocampal complex of the Indian Roller, *Coracias benghalensis*. *Zoology*, *147*, 125929.
Pandey, R. K., & Bhardwaj, S. K. (2016). Effect of differential photostimulation on induction of Fos-like immune-reactivity in the MBH region of Indian weaver bird. *Biological Rhythm Research*, *47*, 641–648.
Patro, N., Kumar, K., & Patro, I. (2013). Quick Golgi method: Modified for high clarity and better neuronal anatomy. *Indian Journal of Experimental Biology*, *51*, 685–693.
Pedersen, A. L., Gould, C. J., & Saldanha, C. J. (2017). Activation of the peripheral immune system regulates neuronal aromatase in the adult zebra finch brain. *Scientific Reports*, *7*, 1-11.
Perez, E. C., Elie, J. E., Soulage, C. O., Soula, H. A., Mathevon, N., & Vignal, C. (2012). The acoustic expression of stress in a songbird: does corticosterone drive isolation-induced modifications of zebra finch calls?. *Hormones and Behavior*, *61*, 573-581.
Prior, N. H. (2014). *The neuroendocrine regulation of pair-maintenance behavior in the opportunistically breeding zebra finch (Taeniopygia guttata)* (Doctoral dissertation, University of British Columbia).
Prior, N. H., & Soma, K. K. (2015). Neuroendocrine regulation of long-term pair maintenance in the monogamous zebra finch. *Hormones and Behavior*, *76*, 11-22.
Ramenofsky, M., & Wingfield, J. C. (2017). Regulation of complex behavioural transitions: migration to breeding. *Animal Behaviour*, *124*, 299-306.
Ramkumar, K., Srikumar, B. N., Venkatasubramanian, D., Siva, R., Rao, B. S., & Raju, T. R. (2012). Reversal of stress-induced dendritic atrophy in the prefrontal cortex by intracranial self-stimulation. *Journal of Neural Transmission*, *119*, 533-543.
Rani, S., Malik, S., Trivedi, A. K., Singh, S., & Kumar, V. (2006). A circadian clock regulates migratory restlessness in the blackheaded bunting, *Emberiza melanocephala*. *Current Science*, *91*, 1093–1096.
Rani, S., Singh, S., Misra, M., Malik, S., Singh, B. P., & Kumar, V. (2005). Daily light regulates seasonal responses in the migratory male redheaded bunting (*Emberiza bruniceps*). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *303*, 541-550.
Ranjan, A., Behari, J., & Mallick, B. N. (2014). Cytomorphometric changes in hippocampal CA1 neurons exposed to simulated microgravity using rats as model. *Frontiers in Neurology*, *5*, 77.
Rao, B. S., Desiraju, T., Meti, B. L., & Raju, T. R. (1994). Plasticity of hippocampal and motor cortical pyramidal neurons induced by self-stimulation experience. *Indian Journal of Physiology and Pharmacology*, *38*, 23-23.
Rastogi, A., Kumari, Y., Rani, S., & Kumar, V. (2011). Phase inversion of neural activity in the olfactory and visual systems of a night-migratory bird during migration. *European Journal of Neuroscience*, *34*, 99-109.
Rastogi, A., Kumari, Y., Rani, S., & Kumar, V. (2013). Neural correlates of migration: Activation of hypothalamic clock(s) in and out of migratory state in the black-headed bunting (*Emberiza melanocephala*). *PLoS ONE*, *8*, 1–12.
Rattenborg, N. C., & Martinez-Gonzalez, D. (2011). A bird-brain view of episodic memory. *Behavioural Brain Research*, *222*, 236–245.

Rosoklija, G., Mancevski, B., Ilievski, B., Perera, T., Lisanby, S. H., Coplan, J. D., Duma, A., Serafimova, T., & Dwork, A. J. (2003). Optimization of Golgi methods for impregnation of brain tissue from humans and monkeys. *Journal of Neuroscience Methods*, *131*, 1-7.

Sadananda, M., & Bischof, H.-J. (2002). Enhanced fos expression in the zebra finch (*Taeniopygia guttata*) brain following first courtship. *The Journal of Comparative Neurology*, *448*, 150–164.

Sadananda, M., & Bischof, H.-J. (2004). c-fos is induced in the hippocampus during consolidation of sexual imprinting in the zebra finch (*Taeniopygia guttata*). *Hippocampus*, *14*, 19-27.

Schweitzer, C., Schwabl, H., Baran, N. M., & Adkins-Regan, E. (2014). Pair disruption in female Zebra finches: consequences for offspring phenotype and sensitivity to a social stressor. *Animal Behaviour*, *90*, 195-204.

Sharma, A., Singh, D., Malik, S., Gupta, N. J., Rani, S., & Kumar, V. (2018). Difference in control between spring and autumn migration in birds: insight from seasonal changes in hypothalamic gene expression in captive buntings. *Proceedings of the Royal Society B*, *285*, 20181531.

Silva-Gomez, A. B., Rojas, D., Juarez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Research*, *983*, 128-136.

Singh, D., Kumari, Y., Rastogi, A., Rani, S., & Kumar, V. (2013). Neuropeptide Y mRNA and peptide in the night-migratory redheaded bunting brain. *Cell and Tissue Research*, *354*, 551–562.

Sköld-Chiriac, S., Nord, A., Tobler, M., Nilsson, J.-A., & Hasselquist, D. (2015). Body temperature changes during simulated bacterial infection in a songbird: fever at night and hypothermia at day. *Journal of Experimental Biology*, *218*, 2961–2969.

Stevenson, T. J., & Kumar, V. (2017). Neural control of daily and seasonal timing of songbird migration. *Journal of Comparative Physiology A*, *203*, 399-409.

Surbhi, Rastogi, A., Malik, S., Rani, S., & Kumar, V. (2016). Seasonal neuronal plasticity in song-control and auditory forebrain areas in subtropical nonmigratory and palearctic-indian migratory male songbirds. *Journal of Comparative Neurology*, *524*, 2914–2929.

Taufique, S. K. T., Prabhat, A., & Kumar, V. (2018). Constant light environment suppresses maturation and reduces complexity of new born neuron processes in the hippocampus and caudal nidopallium of a diurnal corvid: Implication for impairment of the learning and cognitive performance. *Neurobiology of Learning and Memory*, *147*, 120–127.

Templeton, J. J., McCracken, B. G., Sher, M., & Mountjoy, D. J. (2014). An eye for beauty: Lateralized visual stimulation of courtship behavior and mate preferences in male zebra finches, *Taeniopygia guttata*. *Behavioural Processes*, *102*, 33–39.

Thompson, C. K., & Brenowitz, E. A. (2005). Seasonal change in neuron size and spacing but not neuronal recruitment in a basal ganglia nucleus in the avian song control system. *Journal of Comparative Neurology*, *481*, 276-283.

Tomaszycki, M. L., & Adkins-Regan, E. (2005). Experimental alteration of male song quality and output affects female mate choice and pair bond formation in zebra finches. *Animal Behaviour, 70*, 785-794.

Trivedi, A., Rani, S., & Kumar, V. (2005). Differential responses of the photoperiodic clock in two passerine birds possessing a strongly self-sustained circadian system. *Chronobiology International*, *22*, 801-806.

Van Doren, B. M., Liedvogel, M., & Helm, B. (2017). Programmed and flexible: Long-term Zugunruhe data highlight the many axes of variation in avian migratory behaviour. *Journal of Avian Biology*, *48*, 155-172.

Wingfield, J. C. (2013). Ecological processes and the ecology of stress: The impacts of abiotic environmental factors. *Functional Ecology*, *27*, 37–44.

Wingfield, J. C., Pérez, J. H., Krause, J. S., Word, K. R., González-Gómez, P. L., Lisovski, S., & Chmura, H. E. (2017). How birds cope physiologically and behaviourally with extreme climatic events. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160140.

Yadav, A., Kumar, R., Tiwari, J., Kumar, V., & Rani, S. (2017). Sleep in birds: lying on the continuum of activity and rest. *Biological Rhythm Research*, *48*, 805–814.

Yirmiya, R., & Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain, Behavior, and Immunity*, *25*, 181–213.

Zann, R. A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies.* Oxford Univ. Press, 1996.

Papers published / accepted:

Shukla, V., and Sadananda, M. (2021). Social isolation-induced effects on male-specific courtship behaviours and dendritic architecture in associative forebrain areas in Zebra finches (*Taeniopygia guttata*). *Avian Biology Research*, 14, 59-68.

Shukla, V., Rani, S., Malik, S., Kumar, V., and Sadananda, M. (2020). Neuromorphometric changes associated with photostimulated migratory phenotype in the Palaearctic–Indian male redheaded bunting. *Experimental Brain Research*, 238, 2245-2256.

Paper / posters presentations at conferences:

Shukla, V., Malik, S., Rani, S., Kumar, V., and Sadananda, M. Neuronal activity in brain of Red-headed bunting (*Emberiza bruniceps*) during early and late phases of *Zugunruhe* at International Conference on Chronobiology 2021 'Time in the living world' organized by Neuroscience Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru from 15-17 July 2021.

Shukla, V., and Sadananda, M. Social isolation influences courtship behaviours and dendritic remodelling in male Zebra finches (*Taeniopygia guttata*) at 34th Annual meeting of Indian Academy of Neurosciences "Molecules to Mind" held at National Brain Research Centre, Manesar from 19-21 October 2016. Abstract book pg. no. 225.

Shukla, V., Rani, S., Malik, S., Kumar, V., and Sadananda, M. Migratory restlessness induced changes in neuronal activity in Red-headed bunting (*Emberiza bruniceps*) at 26th National Symposium on Chronobiology organized by Department of Studies in Zoology, University of Mysore, Karnataka in association with Indian Society for Chronobiology from 2-3 June 2016. Abstract book pg. no. 45-46.

Shukla, V., and Sadananda, M. Separation-induced changes in courtship behaviours in male Zebra finches (*Taeniopygia guttata*) at IBRO/APRC Varanasi School on Molecular Advancements in Neurobiology held at Banaras Hindu University, Varanasi from 5-20 September 2015, Abstract book pg. no. 57-58.

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